ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Designing Ionic Liquids for the Extraction of Alcohols from Fermentation Broth: Phosphonium Alkanesulfonates, Solvents for Diol Extraction

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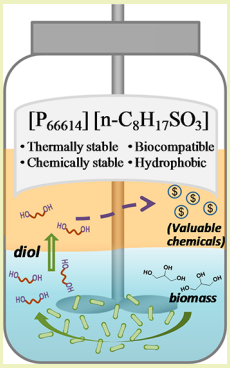
Supporting Information

ABSTRACT: The ability of a number of ionic liquids to extract 1,3-propanediol and 2,3-butanediol from aqueous solution was investigated. A range of hydrophobic ionic liquids were prepared and compared. The long chain phosphonium salts containing the trihexyltetradecyl phosphonium [P_{66614}] cation exhibited promising extraction behavior. Among them, [P_{66614}] [octanesulfonate (C_8SO_3)] combined high hydrophobicity, good stability, and relatively high extraction efficiency with the distribution coefficient D_{BDO} = 0.390 and extraction selectivity S_{BDO} = 4.83 (2,3-butanediol) and D_{PDO} = 0.219 and S_{PDO} = 2.65 (1,3-propanediol) at 25 °C. Additionally, this material exhibited good compatibility with the fermentation process, facilitating its use in bioprocesses.



KEYWORDS: *Ionic Liquids, Renewables, Biocatalysis, 1,3-Propanediol, 2,3-Butanediol*

INTRODUCTION

The application of biocatalysis to the transformation of chemicals, specifically biorenewables, is growing in importance in industry. Major new plants based on fermentation of biomass have been commissioned by several companies (for example, BioAmber, Dupont, and BASF). The fermentation of crude biomass to produce valuable chemicals is regarded as a potential route to replace petroleum products. Whole cell biocatalysis can be highly competitive and less wasteful, and reductions in greenhouse gas emissions have been reported of up to 50%.[1] In order to support this new direction, more effort must be concentrated on the improvement of separations, as the current leading methods of separation of the products of biocatalysis (ion exchange and distillation) are highly energy consuming for products with a significant water solubility and low volatility.

Among the fermentation products, alcohols are attracting much attention, as bioalcohols have a high potential for wide application as biofuels or as chemical feedstocks.[2] Biodiols including 1,3-propanediol (1,3-PDO) and 2,3-butanediol (2,3-BDO) can be produced readily from biomass by fermentation.[3,4] The bottleneck of these processes is the purification procedure, which may comprise 60−70% of the total cost.[5] One alternative way of processing biomass via biocatalysis is to combine biocatalysis and chemocatalysis to form a cascade process; this was proposed by Kieboom in 2002 for enzyme catalysis.[6] Another method employs an ionic liquid (IL) to improve the processing of whole cell biocatalytic processes, such as reduction, oxidation, hydrolysis, and transesterification, where the ILs play the role of distribution of the substrate or product,[7] for example, the extraction of the product from the aqueous phase *in situ*.[8] Previously, we combined these methods and used ILs as media to link whole cell biocatalytic and chemocatalytic steps.[9] Under this method, an ionic liquid is used to extract the intermediate product of biocatalysis from aqueous solution and also as the solvent for downstream chemocatalysis without intermediate separation.[10] On the basis of this concept, the amination and dehydration of 1,3-PDO within an IL were performed.[9,11,12] This method requires a stable ionic liquid that is immiscible with water and exhibits good performance for the extraction of the products of whole cell biocatalysis.

Several bioalcohols can be generated efficiently from biomass via fermentation on a large scale, including ethanol, 1-butanol,[13] 1,3-propanediol,[14,15] and 2,3-butanediol.[16,17] Physical properties of these alcohols are listed in Table 1.

Conventional methods for alcohol recovery center on distillation or vacuum distillation. These processes have a

Received: June 14, 2017
Revised: July 20, 2017
Published: July 27, 2017

DOI: 10.1021/acssuschemeng.7b01934
ACS Sustainable Chem. Eng. 2017, 5, 8260−8268

Table 1. Boiling Points and Polarities of Fermented Alcohols[18]

Chemicals	Boiling point (1 bar)	Polarity, E_T^N (25 °C)
water	100 °C	1.000
1,3-propanediol	214 °C	0.747
ethanol	78 °C	0.654
2,3-butanediol	183−184 °C	0.651
1-butanol	117−118 °C	0.586

high efficiency for ethanol. As the boiling point of the substrates rises, it becomes increasingly expensive to employ distillation. In this case, one of the most promising methods of removal is liquid−liquid extraction.[19] In the extraction, the distribution coefficient ($D_{alcohol}$) and selectivity ($S_{alcohol}$) are two important indices that represent how the alcohol distributes in the two phases and whether the organic solvent extracts the target specifically, which can be calculated by the composition of the two phases (e.g., X_w^{org} stands for the water fraction in the organic phase).

$$D_{alcohol} = \frac{X_{alcohol}^{org}}{X_{alcohol}^{w}} \quad (1)$$

$$S_{alcohol} = \frac{X_{alcohol}^{org}/X_{w}^{org}}{X_{alcohol}^{w}/X_{w}^{w}} \quad (2)$$

Hydrophobic ILs are solvents with a high potential as extractants because of their low vapor pressure and tunable structure.[20] Hydrophobic ILs have been previously studied for the extraction of 1-butanol. 1-Butanol has a comparatively low polarity and is relatively easy to extract with a hydrophobic solvent. Hu et al. adopted 1-(2-hydroxyethyl)-3-methylimidazolium tetrafluoroborate ($[BF_4]^-$) as an extractant and reported good distribution coefficients (D) of about 2.5 at 20 °C. The dissolution of the IL in the water phase was not investigated.[21] Alkylimidazolium-based ILs using the bistriflimide ($[NTf_2]^-$) anion could modify the hydrophobicity affording D_{BuOH} values measured between 1.10 and 1.90.[22−24] Ammonium and phosphonium cations with long alkyl chains show considerable superiority as hydrophobic ions. This could enable the substitution of fluorinated anions for cheaper, more biodegradable, and less toxic alternatives. In 2011, Cascon et al. used $[N_{8888}]$[dihexylsulfosuccinate (DHSS)] and $[P_{66614}]$-[dicyanoamide (DCA)] to extract 1-butanol and achieved a high D_{BuOH} of 7.99 and 7.49, respectively.[25] Garcia-Chavez et al. adopted $[N_{8888}]$[2-methyl-1-naphthoate] and reported a D_{BuOH} of up to 21.[26] Nann et al. studied the anion of tetracyanoborate ($[TCB]^-$) coupled with 3-decyl-1-methylimidazolium and applied it to 1-butanol extraction. The IL exhibited excellent extraction performance with $8.0 < D_{BuOH} < 12.0$.[27] The extraction performance of hydrophobic ILs was further demonstrated by Stoffers et al., who developed a continuous multistep extraction process to extract 1-butanol from aqueous solution with the extractant 1-hexyl-3-methylimidazolium tetracyanoborate. For a total mass flow of 200 g/h, 1-butanol recovery of 85−99% was achieved.[28] The following step of the separation can be simple distillation, and this can be performed under reduced pressure as ILs are nonvolatile or the alcohol in an IL alcohol mixture can proceed downstream for further chemical reaction or processing.

Alcohols of higher polarity, in particular, diols, are more challenging to separate, as their properties are closer to water. For 2,3-BDO and 1,3-PDO, reactive extraction via intermediate ketal formation,[29] salting-out extraction,[30−32] sugaring-out extraction,[33] and continuous extraction within a packed column[34] have been investigated and can give high extraction efficiencies. However, for both of these methods, the contamination of water and the difficulty recovering the additional reagents from water cannot be neglected. So far, most of the liquid−liquid extraction studies targeted toward diols have been based on conventional organic solvents. Malinowski found that hexanal had the best distribution coefficient (D_{PDO} = 0.28) for 1,3-PDO among a range of different alkylalcohols and alkylaldehydes.[35] Escudero et al. used a hydroxy group-based solvent to extract 2,3-BDO, and the highest distribution coefficient obtained was 0.37, which was provided by a 4-nonylphenone/1-decanol co-solvent system.[36] Boonsongsawat et al. designed an ethyl acetate/ethanol co-solvent system to extract 1,3-PDO and achieved a D_{PDO} = 0.31.[37] Anvari et al. studied the *in situ* extraction with oleyl alcohol, resulting in $2.5 < D_{BDO} < 3.0$, which was also found to be biocompatible.[38] The only previous study on hydrophobic ionic liquids was reported by Garcia-Chavez et al. in 2011, who found that $[N_{8888}]$[2-methyl-1-naphthoate] could provide D_{BDO} = 1.06 and S_{BDO} = 11.47 at 40 °C.[26] Compared with the 1-butanol extraction, the extraction of diols was found to be more difficult and has been studied less. Large amounts of extractant were required to achieve high recovery ratios. This reduces the economic feasibility of doing a distillation as a secondary recovery step.

Coupling the separation of the products of biocatalysis to downstream reactions without intermediate isolation could be a profitable way to treat diols formed by fermentation. Ideally the final product, formed by a coupled downstream chemical reaction, will be easier to separate than the bioderived diol.[10] In order to bring together biocatalysis and chemocatalysis into one process, a medium is required that will come into contact with the biocatalysis phase and serve as the solvent for chemocatalysis. There are several requirements for this medium: (1) high hydrophobicity to ensure phase separation, otherwise the reaction medium will dissolve in the biocatalytic phase and require removal and recycle, (2) good extraction ability, (3) high thermal stability to fit the high reaction temperatures commonly required for chemocatalytic reactions to operate at a commercially viable rate, (4) high chemical stability to ensure the reusability of the extractant, and (5) sufficiently low cost to meet industry requirements. In Table 2, the most frequently reported solvents for alcohol extraction are listed, and their key disadvantages are noted with regard to their potential as extraction/*in situ* reaction media.

Table 2. Disadvantages of Previous Research for the Extraction−Reaction System

Extractant	Drawbacks
Conventional solvent (e.g., ethyl acetate and aldehydes)	Low extraction ratio, low thermal stability, high volatility, high flammability
Oleyl alcohol	Hydroxy group functionality that is incompatible with downstream reactions
Alkylimidazolium bistriflimide	Limited extraction ability, water solubility
$[N_{8888}]^+$-based ILs	Low thermal stability
$[TCB]^-$-based ILs	High cost
[2-methyl-1-naphthoate]-based IL	High cost

DOI: 10.1021/acssuschemeng.7b01934
ACS Sustainable Chem. Eng. 2017, 5, 8260−8268

Table 3. Results of 10% 2,3-BDO and 5% 1,3-PDO Solution Extraction with Bistriflimide-Based ILs at 25 °C

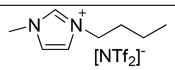
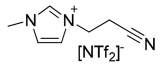
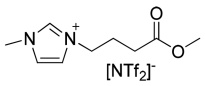
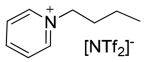
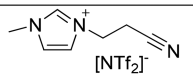
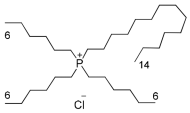
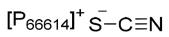
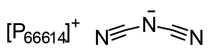
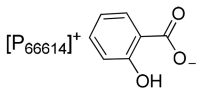
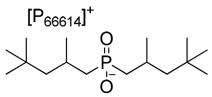
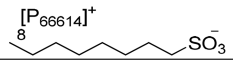
IL	Structure	D_{BDO}	S_{BDO}	D_{PDO}	S_{PDO}	Loss
[BMIM][NTf_2]		0.045	4.37	0.015	1.08	0.6%
[PNMIM][NTf_2]		0.061	2.69	0.024	0.62	2.6%
[BOOMMIM][NTf_2]		0.038	1.31	0.019	0.89	1.7%
[BPy][NTf_2]		0.031	2.33	0.010	0.78	1.0%

Table 4. Results of 10% 2,3-BDO and 5% 1,3-PDO Solution Extraction with $[P_{66614}]^+$-Based ILs at 25°C

IL	Structure	D_{BDO}	S_{BDO}	D_{PDO}	S_{PDO}	Loss
[PNMIM][NTf_2]		0.061	2.69	0.024	0.62	2.6%
[P_{66614}]Cl		0.581	3.57	0.334	2.11	1.28%
[P_{66614}][SCN]		0.197	4.48	0.129	3.06	0.40%
[P_{66614}][DCA]		0.241	7.64	0.095	3.15	0.11%
[P_{66614}][salicylate(Sal)]		0.394	5.93	0.144	2.06	*Trace* [b]
[P_{66614}][diisooctylphosphinate(DIOP)]		0.516	3.32	0.333	2.05	*Trace* [b]
[P_{66614}][1-octane-sulfonate(C_8SO_3)]		0.390	4.83	0.219	2.65	*None* [a]

[a]None means there are no recognized IL peaks on the ^{1}H NMR spectrum of the water phase. [b]Trace means there are trace recognizable IL peaks in the ^{1}H NMR spectrum of the water phase but too small to integrate reliably.

Noting that a perfect solvent system has not yet been found, the investigation reported in this communication was aimed at finding a suitable ionic liquid medium to realize the more efficient combination of biocatalysis and chemocatalysis. Among the requirements, achieving good extraction ability while maintaining hydrophobicity are the hardest to realize, while ionic liquids are considered as the most likely media to merge these two properties together.

RESULTS AND DISCUSSION

Screening of Ionic Liquids. The extraction ability of a range of hydrophobic ionic liquids was evaluated by measuring the extraction of the diols 1,3-PDO and 2,3-BDO from water. The concentration of diol was chosen to correspond with concentrations that are feasible with existing whole cell biocatalysis methods. The desirable properties for the extractant are low cost, low toxicity, high efficiency, and high stability.

The extraction abilities of two commonly used nonvolatile organic solvents, *p*-xylene and dodecane, were first measured. The result showed that the amounts of both 1,3-PDO and 2,3-BDO extracted were low and hard to quantify ($D \approx 0$). This reflects the difficulty extracting these two diols from water in realistic (biologically relevant) concentrations. The design of IL extractants focused on the extraction ability as the first parameter.

^{1}H NMR and Karl Fischer titration were employed to quantify the molar ratio of different components in the two phases. This was found to be more convenient than chromatographic methods.[39] The accuracy of the analysis method was measured using a 400 MHz NMR spectrometer, and errors were less than 1% for the IL phase and less than 2% for the water phase.

For the initial screening of the ILs, simulated fermentation broths were adopted to simplify the extraction system. From the fermentation techniques reported in the literature, 5% of 1,3-PDO solution and 10% of 2,3-BDO are normally achievable via direct fermentation routes without additional concentration.[3,4] The simulated solutions were prepared with these concentrations to mimic the real conditions so that the variation of extraction as a result of ionic liquid functionality could be studied and rationalized.

Hydrophobic ILs most commonly employ the bistriflimide anion ($[NTf_2]^-$), which confers high hydrophobicity, low viscosity, and low melting point.[40] The alkylimidazolium and alkylpyridinium structures were chosen as common cations of reasonable cost and flexibility. The central structures were functionalized with various groups. The extraction performance of four representative ILs are reported in Table 3.

From Table 3, the extraction performances of all the bistriflimide-based ILs screened were very limited. $[BMIM]^+$,

DOI: 10.1021/acssuschemeng.7b01934
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$[BPy]^+$, and $[BOOMMIM]^+$ could only provide $D_{2,3\text{-BDO}} < 0.05$ and $D_{1,3\text{-PDO}} < 0.02$. The $[PNMIM]^+$ gave a distribution coefficient 33% higher than $[BMIM]^+$, but this was accompanied by an increased loss of IL to the aqueous phase. Because of the high cost of the extractant, especially for the bistriflimide anion, any loss of IL could not be accepted. For this reason, none of the imidazolium ILs tested were deemed appropriate media.

Another route to the design of IL media for the extraction of alcohols from aqueous solution is to adopt a hydrophobic cation. Both long chain (>C12) ammonium and long chain phosphonium ILs are relatively economical options, while, in general, phosphonium ILs are more chemically and thermally stable. Trihexyltetradecylphosphonium ($[P_{66614}]^+$) has an asymmetric structure, leading to ILs with a low melting point and relatively low viscosity. $[P_{66614}]Cl$ (commercially available from SOLVAY) is a convenient precursor for a range of ILs via anion exchange.

From Table 4, the long chain phosphonium-based ILs, coupled with different anions, presented much higher extraction abilities than the bistriflimide ILs. Among all of the phosphonium ILs, $[P_{66614}]Cl$ gave the best distribution coefficient, but it was slightly soluble in water, which will restrict application. $[P_{66614}][DCA]$ exhibited the highest extraction selectivity among the ILs tested, but the distribution coefficient was low for both diols targeted. $[P_{66614}][DIOP]$, $[P_{66614}][Sal]$, and $[P_{66614}][C_8SO_3]$ provided reasonable extraction performance ($D_{BDO} > 0.35$, $D_{PDO} > 0.10$) and nearly zero loss of extractant. Comparing these three ILs, $[P_{66614}][DIOP]$ had the highest D_{BDO} and D_{PDO} but attracted a large amount of water, which caused the low extraction selectivity. Meanwhile, even though $[P_{66614}][DIOP]$ was very hydrophobic, an interphase layer was formed during the stirring process, which could not be avoided or eliminated, which led to trace IL loss (Figure 1). In contrast, $[P_{66614}][C_8SO_3]$ and

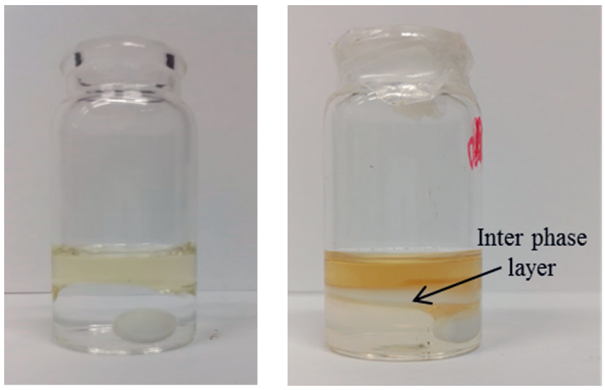

Figure 1. IL/water phase behavior of $[P_{66614}][C_8SO_3]$ and $[P_{66614}]$-[DIOP].

$[P_{66614}][Sal]$ showed better extraction selectivity but lower distribution coefficients. They behaved similarly for 2,3-BDO (D_{BDO}), while $[P_{66614}][C_8SO_3]$ gave a D_{PDO} 50% higher than $[P_{66614}][Sal]$.

Stability of ILs. The stability of ILs should also be considered before they are applied to integrated processes and long-term use. Among the three ILs, $[P_{66614}][DIOP]$, $[P_{66614}]$-[Sal], and $[P_{66614}][C_8SO_3]$, only $[P_{66614}][C_8SO_3]$ exhibited satisfactory thermal stability. As analyzed by thermal gravimetric analysis (Figure S5), the decomposition temperature of $[P_{66614}][C_8SO_3]$ was found to be higher than 350 °C. Because of the relatively poor stability of phosphinate and carboxylate anions, $[P_{66614}][DIOP]$ and $[P_{66614}][Sal]$ presented lower decomposition temperatures of 219 and 292 °C, respectively, although the cations of $[P_{66614}]^+$ are highly stable.[41] As the conjugated anion of a strong acid, 1-octanesulfonate is hard to protonate and should be stable in the presence of metabolic acids, such as acetic acid, butyric acids and L-lactic acid, as the conjugate anions of weak acids salicylate and phosphinate are more susceptible to chemical decomposition via protonation. In summary, $[P_{66614}][C_8SO_3]$ was chosen as the superior extraction medium for biodiols, considering the extraction performance and stability.

Biocompatibility. If an ionic liquid is to be used in a biocatalytic process, the biocompatibility of the IL is one of the key factors for consideration. A general trend of anion toxicity can be extrapolated from literature studies. When coupled with the same cation (e.g., alkylimidazolium), the trend of decreasing anion toxicity has been reported as $[NTf_2]^- \geq [PF_6]^- > [CH_3SO_4]^- > [BF_4]^- > [OTf]^- > [CH_3SO_3]^- > Br^- \approx Cl^-$.[42−44] In terms of the cation, it was found the imidazolium cation with a shorter alkyl chain is relatively nontoxic, while the $[P_{66614}]^+$-based ILs have similar toxicity to imidazolium salts.[45] In this study, the most promising three ILs for extraction, $[P_{66614}][DIOP]$, $[P_{66614}][Sal]$, and $[P_{66614}]$-$[C_8SO_3]$, were analyzed by a biocompatibility assay with the bacterium *Clostridium butyricum*, which can be employed to generate 1,3-PDO from glycerol.[9] The initial study was a cell viability assay. The growth rates of the bacterium were measured in the presence of the ionic liquids and compared with the growth rate in the absence of IL (Figure 2).

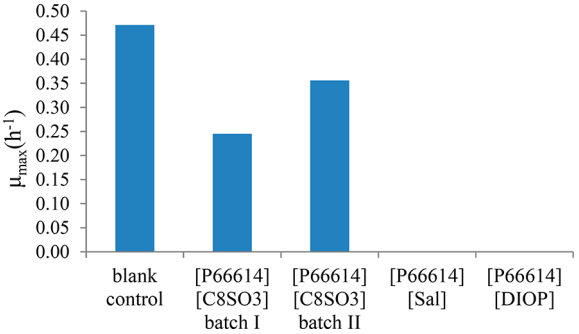

Figure 2. Maximal specific growth rates for microorganism *C. butyricum* with different ILs (speed: 120 rpm, temp.: 35 °C, IL loading: 25 v%).

The maximal specific growth rate (μ_{max}) indicates the multiplication rate of the microorganism, which reflects the adaptation of bacteria to the environment. According to Figure 2, the highest maximal specific growth rates (μ_{max}(average) = 0.30 ± 0.015 h^{-1}) was enabled by $[P_{66614}][C_8SO_3]$, while the other two ILs, $[P_{66614}][Sal]$ and $[P_{66614}][DIOP]$ showed total inhibition of bacterial growth. The use of $[P_{66614}][C_8SO_3]$ reduced the growth by 36% compared to the blank control experiment. Cornmell et al.[46] found that when [trioctylmethylammonium][NTf_2] and $[P_{66614}][NTf_2]$ were presented with the broth of *Escherichia coli*, the cultivation of the bacteria was partly inhibited, but the whole cell catalysis process was greatly improved by more than 200%. One explanation for the variation across the different ILs is that the biocompatibility was influenced by the solubility of the IL. It is

DOI: 10.1021/acssuschemeng.7b01934
ACS Sustainable Chem. Eng. 2017, 5, 8260−8268

known that the phosphonium cation can be toxic to bacterial cells.[47] From the extraction experiments, although $[P_{66614}][Sal]$ and $[P_{66614}][DIOP]$ were regarded as insoluble in water, there was still recognizable but nonintegrable IL peaks in the ^1H NMR spectra of the water phase. According to the equipment and analysis method, the solubility of these ILs in water was found to be lower than 0.1 mg/g. This limited solubility nevertheless led to the exposure of the cells to the ILs. $[P_{66614}][C_8SO_3]$ was the only suitable IL examined. The influence of this IL on the bacterial growth was further evaluated (Figure 3).

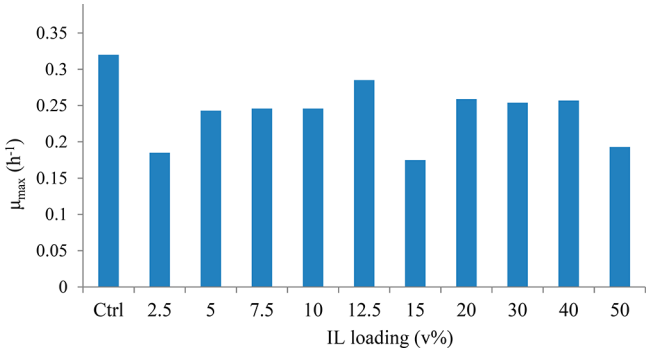

Figure 3. Maximal growth rates for *C. butyricum* with different loadings of $[P_{66614}][C_8SO_3]$ (speed: 120 rpm, temp.: 35 °C).

The average growth of the bacteria in the presence of the IL was 0.23 h^{-1}, reduced by 28% compared with the control experiment. However, overviewing the full range of IL loading experiments, there was no obvious trend with the loading of the IL. As the fermentation process was influenced by many factors, the variation of the fermentation result was relatively large. Thus, the difference among the growth rates could be considered as the systemic error. On the basis of this acknowledgment, it can be concluded the IL had a negative effect on the growth of the bacterium; however, the extent of inhibition did not vary with the amount of the IL added. The concentration of each organic component of the final mixture was also measured (Figure 4).

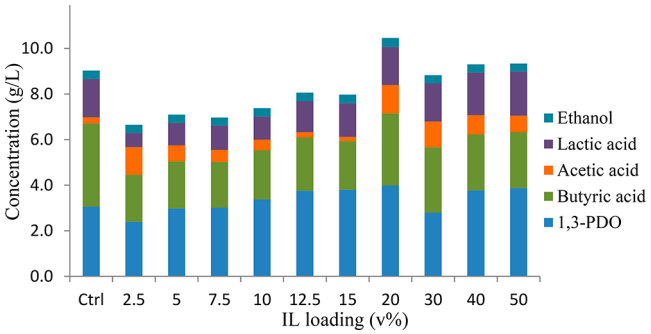

Figure 4. Concentration of different products with different loadings of $[P_{66614}][C_8SO_3]$ (speed: 120 rpm, temp.: 35 °C).

Figure 4 shows how the final concentrations of different products varied with different amounts of IL added. In terms of the 1,3-PDO yield, most of the experiments achieved better results than the control experiment. It was found that the presence of the IL could enhance the production of the desired biochemicals, even though the growth of the bacterium was inhibited, which was consistent with the results of Cornmell et al.[46] Due to the relatively low distribution coefficient of 1,3-PDO in the IL/water system, 1,3-PDO analyzed in the aqueous phase reflected the yield. However, for other major products like butyric acid, the control experiment provided the highest concentration, which was because of the larger distribution coefficient of the butyric acid in the biphase system. Therefore, the yield of butyric acid was underestimated by an amount that was dependent on the volume of the IL phase.

In summary, $[P_{66614}][C_8SO_3]$ had good biocompatibility and was found to promote the biocatalytic activity of *C. butyricum* to generate 1,3-PDO. The *in situ* extraction can be followed by an *in situ* catalytic route to convert 1,3-PDO to volatile products, e.g., propanal, which also realizes the recovery of the IL.[12] In this way, this IL can facilitate the combination of biocatalysis and chemocatalysis, playing the role of the intermediate phase for two *in situ* processes.

Extraction of Fermentation Broth. The composition of fermentation broths resulting from whole cell biocatalysis is very variable and depends on many factors, including the microbe employed, the precise conditions, any impurities in the substrate, and the method of delivery of the substrate into solution. A fermentation broth prepared by a fed-batch process employing *C. butyricum* and separated from particulates by centrifugation was subjected to extraction by $[P_{66614}][C_8SO_3]$. A broth was chosen that contained a range of typical co-products and some residual glycerol in order to quantify the extraction of each organic component. In addition to the organic products of metabolism, fermentation broths contain inorganic salts, protein, and cell fragments; these can be removed by ion exchanging column and centrifugation.

The extraction of the fermentation broth of 1,3-PDO was conducted in a three-step operation with the total IL loading of 2:1 over the fermentation broth (Table 5). Starting with the

Table 5. Extraction of Real Fermentation Broth of 1,3-PDO at 25 °C

	1,3-PDO	Glycerol	Acetic acid	Lactic acid	Butyric acid
Concentration in the real broth (g/L)	50.8	1.2	1.5	10.8	11.0
Extraction ratio	41%	17%	46%	63%	75%

1,3-PDO concentration of 50.8 g/L, 41% of the diol was transferred to the organic phase. Acetic acid, lactic acid, and butytic acid were also extracted with higher recovery ratios. The extraction ratio of the substrate glycerol was much less than that of the other organic compounds. This shows that, working at high conversion in a fed batch mode, a significant quantity of the products of microbial metabolism can be extracted, while leaving sufficient substrate behind for the bacteria to digest. The efficiency of the separation increases as the polarity of the product reduces.

The extracting solvent is not water soluble and does not enter the aqueous phase. Avoiding the addition of any water-soluble reagents, such as sugar or salt, means that the aqueous phase is cleaned up by the extraction process. This is important when considering the overall sustainability of the process as ultimately water must be recycled back to the fermentation.

Ternary Liquid–Liquid Equilibria. The ternary liquid–liquid equilibrium (LLE) or ternary phase diagram presents the solute and solvent distribution (Figures 5 and 6); from this, the phase and extraction behavior of every starting composition can

DOI: 10.1021/acssuschemeng.7b01934
ACS Sustainable Chem. Eng. 2017, 5, 8260–8268

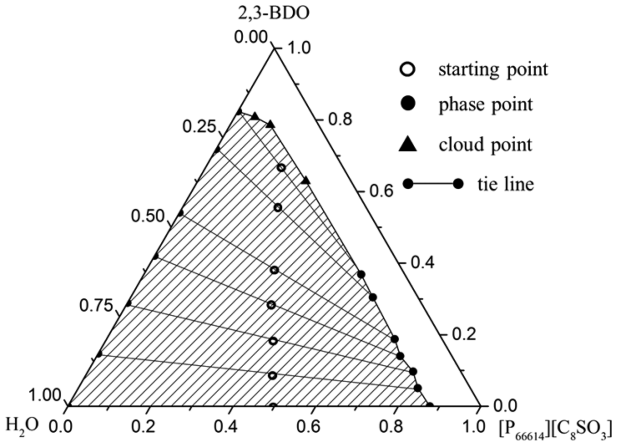

Figure 5. Ternary LLE of water/2,3-BDO/$[P_{66614}][C_8SO_3]$ system at 25 °C.

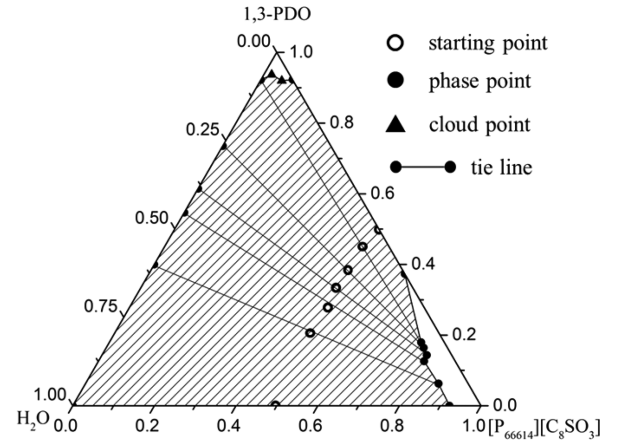

Figure 6. Ternary LLE of water/1,3-PDO/$[P_{66614}][C_8SO_3]$ system at 25 °C.

be read. The ternary LLE diagrams of water/diols/$[P_{66614}][C_8SO_3]$ were assayed by preparing mixtures of the three components with a series of compositions and analyzing the composition of the two separated phases. For the critical phase points for which it was hard to get clear phase separation, the measurement of the cloud point was used instead. The LLE data was calculated by weight for each point presented.

In the LLE diagram, the shadowed area indicates the biphasic region, where the boundary was delineated with the phase points, and the tie lines express the distribution of the diols in the two phases. For the LLE diagrams of water/diol/$[P_{66614}][C_8SO_3]$, it can be found that all of the points of the water-rich phase are located on the axis, which means the content of the IL was zero in the water phase. Comparing the two LLE diagrams, the one with 2,3-BDO contains a larger single phase area and higher slope of the tie lines, expressing a higher extraction efficiency of 2,3-BDO.

EXPERIMENTAL SECTION

Material and Equipment. All the chemicals used in this project, if not specially stated, are the purest available commercial products from Sigma-Aldrich: *n*-dodecane, *p*-xylene, 1,3-propanediol, 2,3-butanediol, 1-methylimidazole, pyridine, 1-bromobutane, 1-chloropropionitrile, methyl 4-chlorobutyrate, sodium thiocyanate, lithium bistriflimide (3M), sodium 1-octanesulfonate (TCI, > 98%), trihexyl(tetradecyl) phosphonium chloride (Cytech, 97.7%), trihexyl(tetradecyl) phosphonium bis-2,4,4-(trimethylpentyl)phosphinate (Cytech, > 95.0%), and trihexyl(tetradecyl)phosphonium dicyanamide (Cytech, > 95.0%).

The Y5 media composition used for bacterial growth is as follows: 20 g of glycerol (Centralchem, > 99%), 3 g of yeast extract (BioSpringer), 5 g of potassium phosphate (Mikrochem), 5 g of dipotassium phosphate (Mikrochem), 0.01 g of cobalt(II) chloride hexahydrate (Centralchem, > 99%), and 3 g of acetic acid (Centralchem, 98%). The pH was adjusted to 7 by ammonia solution (Centralchem, 25−27%), sparged with N_2 for 15 min at laboratory temperature, and then autoclaved at 120 kPa, 121 °C for 20 min. A total of 0.2 g of magnesium sulfate heptahydrate (Centralchem, 99%) was dissolved in 10 mL of demineralised water and autoclaved separately, and 0.01 g of iron(II) sulfate heptahydrate was added to autoclaved Y5 media through a bacterial filter, Filtropur, with a porosity of 0.2 μm (Sarstedt, Germany).

A hot plate stirrer (Heidolph, Pt-1000), centrifuge (Eppendorf, 5702), vacuum pump (Edwards RV8), rotary evaporator (BUCHI Rotavapor R-114), microplate reader (Varioscan Flash, Fisher Scientific, USA), anaerobic chamber (Bactron I, Shel Lab, USA), 96-well microplates (Sarstedt Microtest Plate 96 Well), Durham bottles (Fisherbrand, Fisher Scientific, USA), NMR (Bruker, 400 MHz), Karl Fischer (899 Coulometer), mass spectrometer (Waters Micromass LCT Premier Mass Spectrometer), CHNS (PerkinElmer, 2400 Series II), TGA (PerkinElmer, Q5000), HPLC (Agilent Technologies 1220 Infinity LC) were used.

IL Synthesis. *Synthesis of Bistriflimide-Based ILs.*[48] 1-Methylimidzole (or pyridine) (0.1 mol) was dissolved in 30 mL of acetonitrile and then mixed with alkylating reagent (~0.12 mol). The reaction was carried out in acetonitrile under reflux. The reaction lasted at least 12 h and was followed by ^{1}H NMR by monitoring the conversion of the ring structure until 1-methylimidaozle (or pyridine) was fully converted. The solvent and alkylation reagent was removed by rotavap and high vacuum to obtain the halide salt. The halide was dissolved in deionized water (30 mL) with continued stirring for 2 h after adding Li$[NTf_2]$ (0.12 mol). The oil phase was washed with Li$[NTf_2]$ solution (1.0 mol/L, 30 mL) once and deionized water (30 mL) twice. The last filtrate was tested with acidic $AgNO_3$ solution to confirm no halide residue. The wet product was dried at 80 °C under high vacuum.

Synthesis of $[P_{66614}]^+$-Based ILs.[49] $[P_{66614}]$Cl (0.05 mol) was dissolved in acetone (50 mL), and sodium 1-octanesulfonate (0.05 mol) was added in another aliquot of acetone (50 mL) to make a slurry. The two portions were mixed together and maintained stirring for 48 h to ensure sufficient anion exchange. After stirring, the NaCl precipitate was removed by centrifuge at 4400 rpm for 5 min. The acetone was removed by rotary evaporation and high vacuum to yield the crude product. The hydrophobic IL was further purified by washing with 20 mL of deionized water three times. Lastly, the product was completely dried under high vacuum at 80 °C.

3-(4-Methoxy-4-oxobutyl)-1-methylimidazolium Bistriflimide ([BOOMMIM][NTf$_2$]).[48] Product: 34.58 g, yield: 74.7%. ^{1}H NMR (400 MHz, CD_3CN): δ = 8.45 (s, 1H, N═CHN), 7.41 (t, J = 1.6 Hz, 1H, C═CHNMe), 4.37 (t, J = 7.2 Hz, 2H, $CH_2(CH_2)_2OH$), 4.20 (t, J = 7.2 Hz, 2H, NCH_2C), 3.859 (s, 3H, NCH_3), 3.66 (s, 3H, $COOCH_3$), 2.37 (t, J = 7.2 Hz, 2H, CCH_2CO), 2.13 (m, 2H, CH_2CH_2CO) ppm. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 172.99 (O═C-O), 136.62 (N═CHN), 123.82 (MeNC═CH), 122.35 (MeNC═C), 119.76 (q, C−F coupling, J = 320.98 Hz, CF_3), 51.68 (NCH_2C), 48.70 (NCH_3), 36.61 ($COOCH_3$), 30.17 (CCH_2CO), 25.13 (CH_2CH_2CO) ppm. ^{19}F NMR (282 MHz, CD_3CN): δ = −79.77 ppm. Mass spectrum (TOF ES+) m/z: 183.11 (calc. for $[M-[NTf_2]^-]^+$: 183.23), 646.14 (calc. for $[2M-[NTf_2]^-]^+$: 646.60). Elemental analysis (%): Calc. for $C_{11}H_{15}F_6N_3O_6S_2$: C, 28.51; H, 3.26; N, 9.07; S, 13.84. Found: C, 28.62; H, 3.32; N, 9.06; S, 13.70.

Trihexyltetradecylphosphonium 1-Octanesulfonate $[P_{66614}][C_8SO_3]$. Product: 32.80 g, yield: 96.9%. ^{1}H NMR (CD_3CN): δ = 2.52 (t, J = 8.0 Hz, 2H, CH_2SO_3), 2.07 (m, 8H, PCH_2), 1.68 (m, 2H, CH_2CH_2SO3), 1.6−1.3 (m, 58H, $CH_2(CH_2)_nCH_3$), 0.93 (m, 15H, CH_3) ppm. ^{31}P NMR (161 MHz, CD_3CN): δ = 33.53 ppm. Mass spectrum (TOF ES+) m/z: 483.50 (calc. for $[P_{66614}]^+$: 483.86),

DOI: 10.1021/acssuschemeng.7b01934
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1160.11 (calc. for $[M+[P_{66614}]^+]^+$: 1161.00); (TOF ES-) *m/z*: 193.09 (calc. for $[C_8SO_3]^-$: 193.28), 869.70 (calc. for $[M+[C_8SO_3]^-]^-$: 870.42). Elemental analysis (%): Calc. for $C_{40}H_{85}O_3PS$: C, 70.95; H, 12.65; S, 4.74. Found: C, 70.56; H, 12.85; S, 4.72.

$[P_{66614}][C_8SO_3]$ is reported for the first time; closely related $[P_{66614}][1\text{-octanesulfate}(C_8SO_4)]$ was reported previously (CAS: 1361552-10-4).[49]

Liquid–Liquid Extraction. Glass sample vials (15 mL) were used as the vessels for extraction (Figure 1). The temperature was controlled using a water bath. The simulated broths were prepared in advance with the initial concentration of 5 wt % 1,3-PDO and 10% 2,3-BDO, which were based on realistic fermentation broths. The loading of the two phases was 2g of IL and 3 g of aqueous solution. The biphasic system was stirred at a high speed (1000 r/min) for more than 5 h. After extraction, the emulsion stood in the water bath for another 5 h to ensure complete phase separation. After the extraction, the two phases were carefully separated using glass pipettes. Duplicate or triplicate experiments were carried out.

After the two phases were completely separated, 2 drops of the sample were transferred into the NMR tube, and 0.5 mL of CD_3CN was added. The analysis was carried out on a 400 MHz NMR spectrometer.

In the multistep extraction of real fermentation broth, each single step of the extraction followed the same procedure and loading ratio as the modeling experiments. After the phase separation and sampling, another portion of fresh IL was added into the vial for another extraction step.

Assay of Liquid–Liquid Equilibrium Diagram. Each component was weighed accurately in the desired composition comprising a total mass of about 2 g. The sample was loaded in a GC vial with a stirring bar. The mixture was stirred for 24 h within a temperature controlled at 25 °C by the water bath. After that, the sample was centrifuged at 4400 rpm for 15 min to achieve clear phase separation. The analysis of the two phases was the same as that for the extraction experiments.

For measuring cloud points, the diol and IL were weighed and loaded into the GC vial first. The vial was placed in a temperature controlled water bath and stirred continuously. Then, water was added into the vial in 20 μL aliquots using a micropipette. After adding the water each time, the solution was observed with a magnifying glass to check if the microdrops of water had disappeared. This step was repeated until the solution turned cloudy and the microdrops did not vanish in 5 min. The water amount was counted by volume.

Biocompatibility Test Performance. Microbial strain *C. butyricum* DSM 4278 used in this study was stored on Y5 medium agar plates in an anaerobic chamber with an inert atmosphere (90% N_2, 5% CO_2, 5% H_2) at 34 °C. The stock culture was replated every 2 weeks. Individual bacteria colonies were used for inoculum preparation in 25 mL Durham bottles containing 10 mL of Y5 medium. Inoculum was cultivated for 9–12 h at 34 °C with a magnetic stirrer at 220 rpm in an anaerobic chamber until the optical density at 600 nm reached 6.0–7.0.

Cultivations for biocompatibility tests were performed in 96-well microplates with a working volume of 200 μL (inoculation 5 v%) measured by a microplate reader at 35 °C, speed of 120 spm, and diameter of 4 mm. Preparations of the microplate and its inoculation were performed in an anaerobic chamber, and after the covering of the microplate by a permeable membrane (Breathe-Easy, Sigma-Aldrich), it was placed in the microplate reader to start an experiment.

Glycerol, 1,3-PDO, and acids were analyzed by HPLC (Agilent Technologies 1220 Infinity LC) on a Polymer IEX H+ form 8 μm, 250 mm × 8 mm column (Watrex) with an attached Polymer IEX H+ form 8 μm, 40 mm × 8 mm guard column (Watrex) at 50 °C. The column was attached to an RI detector (Agilent Technologies 1260 Infinity) and UV detector (at 258 nm). The mobile phase used 9 mM H_2SO_4 at a flow rate of 1.05 mL/min.

CONCLUSIONS

Compared with bistriflimide-based hydrophobic ILs, $[P_{66614}]^+$ ILs exhibited much better diol extraction performance. $[P_{66614}][DIOP]$, $[P_{66614}][Sal]$, and $[P_{66614}][C_8SO_3]$ provided the most efficient extraction. $[P_{66614}][C_8SO_3]$ was found to have a greater thermal stability and a more stable anion and therefore a greater utility. At 25 °C, the extraction coefficients of $[P_{66614}][C_8SO_3]$ were found to be $D_{BDO} = 0.390$, $S_{BDO} = 4.83$ and $D_{PDO} = 0.219$, $S_{PDO} = 2.65$. $[P_{66614}][C_8SO_3]$ also exhibited good biocompatibility with *C. butyricum*. When added into the fermentation process, the cultivation of the bacteria was partly inhibited, but the desired biocatalytic process was enhanced. As 1-octanesulfonate is a readily available and commonly used anion, the cost of this IL is much cheaper than many of the ILs reported in the literature, such as those containing tetracyanoborate ($[TCB]^-$)[28]- and 2-methyl-1-naphthoate[26]-based ILs. In summary, $[P_{66614}][C_8SO_3]$ was found to be an ideal solvent for the extraction of diols from fermentation broth.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b01934.

^{1}H NMR spectra of ionic liquids and extraction mixtures, preparation of cell suspensions, TGA of ionic liquids, tables of phase compositions. (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by Queen's University Ionic Liquids Laboratories (QUILL), China Scholarship Council (X. Liu), and GRAIL (Grant Agreement No. 613667), project cofinanced by the European Commission under the 7th Framework Programme. This work was cofunded by the Slovak Research and Development Agency under Contract No. DO7RP-0045-12. The analytical assistance from QUILL and Analytical Services and Environmental Projects Division (ASEP) in Queen's University Belfast is also acknowledged.

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